Chemosphere 202 (2018) 410–419

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Ciprofloxacin *vs.* temperature: Antibiotic toxicity in the free-floating liverwort *Ricciocarpus natans* from a climate change perspective

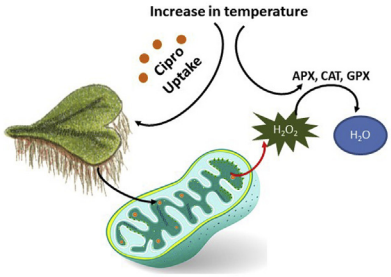
Marcelo Pedrosa Gomes [a, b, *], Júlio César Moreira de Brito [c, d], Elisa Monteze Bicalho [b], Janaína Guernica Silva [b], Maria de Fátima Gomides [c], Queila Souza Garcia [b], Cleber Cunha Figueredo [b, **]

[a] Universidade Federal do Paraná, Setor de Ciências Biológicas, Departamento de Botânica, Avenida Coronel Francisco H. dos Santos, 100, Centro Politécnico Jardim das Américas, C.P. 19031, 81631-980 Curitiba, Brazil
[b] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Botânica, Avenida Antônio Carlos, 6627, Pampulha, Caixa Postal 486, 31270-970 Belo Horizonte, Minas Gerais, Brazil
[c] Fundação Ezequiel Dias, Rua Conde Pereira Carneiro, 80, Belo Horizonte, 30510-010 Minas Gerais, Brazil
[d] Programa de Pós-Graduação em Inovação Tecnológica e Biofarmacêutica, UFMG, Minas Gerais, Brazil

## HIGHLIGHTS

- Increased temperature favored Cipro uptake by *R. nattans*.
- Rising temperature increase Cipro-inducing ROS formation by mitochondria.
- Rising temperature favors the activity of antioxidant enzymes.
- Temperature can prevent deleterious effects of Cipro by preventing oxidative stress.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 14 November 2017
Received in revised form
8 February 2018
Accepted 7 March 2018
Available online 19 March 2018

Handling Editor: Jian-Ying Hu

*Keywords:*
Antibiotic
Fluoroquinolone
Oxidative stress

## ABSTRACT

The physiological responses of the aquatic liverwort *Ricciocarpus natans* to ciprofloxacin (Cipro) exposure under different growth temperatures were investigated. Cipro appears to act as an inhibitor of mitochondrial Complex III by blocking the oxidation of quinol, resulting in the formation of hydrogen peroxide ($H_2O_2$). $H_2O_2$ accumulation upon Cipro exposure is responsible for decreased photosynthesis in plants. The amount of $H_2O_2$ in plants is kept under control by antioxidant enzymes, whose activities are central to the responses of plants to Cipro yet are influenced by temperature. Increased temperature favored Cipro uptake by plants as well as its deleterious effects on mitochondrial activity; however, it also favored the activity of antioxidant enzymes, thereby preventing the exacerbation of the deleterious effects of Cipro. The uptake of Cipro by plants appears to be largely a passive process, although some uptake must be driven by an energy-consuming process. *Ricciocarpus natans* should be considered for programs aimed at the reclamation of Cipro since this plant exhibits high Cipro-tolerance, the capacity

*Abbreviation:* APX, ascorbate peroxidase; CAT, catalase; Cipro, ciprofloxacin; CMS, cell membrane stability; ETC, electron transport chain; ETR, electron transport rate; *Fv/Fm*, the maximal PSII photochemical efficiency; GPX, guaiacol peoxidase; $H_2O_2$, hydrogen peroxide; MDA, malonodialdehyde (lipid peroxidation); NPQ, non-photochemical quenching; PS, photosystem; ROS, reactive oxygen species; Qo, quinoloxidation; qP, photochemical quenching; UQ, oxidized ubiquinone; $UQH_2$, reduced ubiquinone; $UQF_{rel}$, the relative unquenched fluorescence.

* Corresponding author. Universidade Federal do Paraná, Setor de Ciências Biológicas, Departamento de Botânica, Avenida Coronel Francisco H. dos Santos, 100, Centro Politécnico Jardim das Américas, C.P. 19031, 81631-980 Curitiba, Brazil.
** Corresponding author.
*E-mail addresses:* marcelo.gomes@ufpr.br (M.P. Gomes), clebercf@icb.ufmg.br (C.C. Figueredo).

https://doi.org/10.1016/j.chemosphere.2018.03.048

Photosynthesis
Respiration
Toxicity

for accumulation and increased uptake rates of the antibiotic with increasing temperatures (from 20 to 30 °C).



## 1. Introduction

Contamination of freshwater environments by hazardous compounds is a global concern. In a recent survey of 38 US streams, bioactive-anthropogenic-organic compounds, such as pharmaceuticals (including antibiotics) comprised about 57% of the targeted organic analytes detected in the water (Bradley et al., 2017). Among the observed antibiotics, ciprofloxacin [Cipro; 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dyhrydroquinoline-3-carboxylic acid] was found in 26% of the studied sites (Bradley et al., 2017). Cipro is a fluoroquinolone commonly used in human and veterinary medicine for prophylaxis or treatment of bacterial diseases (Migliore et al., 2003). Aquatic systems are the main sink for antibiotics (mainly from agricultural sites) and significant concentrations of Cipro ranging from μg to mg $l^{-1}$ have been reported in environmental matrices (Golet et al., 2003; Martínez-Carballo et al., 2007; SFT, 2007; McClellan and Halden, 2010; Bradley et al., 2017). Cipro is quite resistant to abiotic and biotic degradation (Girardi et al., 2011), and its persistence in an environment may result in detrimental effects to trophic systems and the induction of Cipro-tolerance in target bacteria.

In target pathogenic bacteria, Cipro acts by inhibiting DNA replication and repair (Brighty and Gootz, 2000). However, observed detrimental effects of the antibiotic in non-target organisms, such as animals, including humans, and plants (Lawrence et al., 1996; Kozieł et al., 2006; Lowes et al., 2009; Gomes et al., 2017a) indicate that Cipro could have other mechanisms of action. Due to its chemical similarity with quinones, Cipro was initially proposed to act as a quinone-site inhibitor in PSII of photosynthesizing organisms (Aristilde et al., 2010). This hypothesis, however, was subsequently criticized and Cipro was proposed to have a mechanism of interference with photosynthesis other than binding to quinone sites in the chloroplast electron transport chain (ETC) (Aristilde et al., 2010; Gomes et al., 2017a). According to Aristilde et al. (2010), Cipro interferes with the electron transference from chlorophylls in the antenna complex to the reaction center in RC-II, resulting in delays in the photoreduction of the primary quinone acceptor. On the other hand Gomes et al (2017a), proposed that Cipro leads to the overproduction of reactive oxygen species (ROS) which, in turn, promote oxidative stress, such as damage to thylakoid membranes and suppression of *de novo* synthesis of PSII-associated proteins, resulting in decreased photosynthetic activity.

The relatively rapid changes in environmental temperatures due to anthropogenic disturbances over the past century have affected aquatic habitats (Knutti et al., 2016). Fluctuations in temperature are known to alter the effects of chemical contaminants in aquatic organisms and the effects of each stressor (contaminant and temperature) alone may be attenuated or exacerbated by their interactions (Folt et al., 1999; Chalifour and Juneau, 2011; Gomes and Juneau, 2017). In a context of climatic change, it is essential to understand how variation in environmental conditions will modify the toxicity of water contaminants (Gomes and Juneau, 2017). For instance, Cipro and temperature are two environmental factors that can affect photosynthesis and respiration (Bicalho et al., 2017; Gomes et al., 2017a), but the literature is not clear on if and how these factors can interact in affecting photosynthesizing organisms. Moreover, temperature modulates the activities of antioxidant systems (Sobrino and Neale, 2007; Bicalho et al., 2017) that are needed to counteract the ROS accumulation induced by Cipro exposure in plants (Gomes et al., 2017a). Temperature might, therefore, interact antagonistically, additively or synergistically with Cipro, modulating its effects on cellular mechanisms.

Changes in membrane composition and adjustments of membrane fluidity are also common responses of plants to thermal acclimation (Falkowski and Raven, 2013) and are related to the uptake of contaminants from water (Gomes and Juneau, 2017). Therefore, temperature changes have important applied implications with respect to the toxicity of chemicals in aquatic systems and their removal (Gomes and Juneau, 2017). The cellular mechanism of Cipro uptake is still not clear; it may be a process of passive diffusion, as proposed by some authors (Bedard et al., 1987; Chapman and Georgopapadakou, 1988; Cohen et al., 1988), or an energy-dependent active transport mechanism, as postulated by Diver et al. (1990). Nonetheless, changes in temperature can result in modulation of both passive and active process of Cipro uptake by plants.

The free-floating aquatic liverwort *Ricciocarpus natans* is recognized for its ability to accumulate contaminants directly from water (Chiodi Boudet et al., 2011; Sharma and Sachdeva, 2015). Bryophytes have little or no developed cuticle, which is associated with their ability to retain contaminants since chemicals on the surface of cells have direct access to their absorption sites (Sharma and Sachdeva, 2015). Moreover, the higher protein:fiber ratios of the cell walls of bryophytes may account for their great capacity to reclaim water contaminants (Sharma and Sachdeva, 2015). To our knowledge, however, there are no studies regarding the mechanism by which aquatic liverworts uptake antibiotics, and how changes in growth temperature may affect this process.

Here, we investigated the responses of the aquatic liverwort *R. natans* to Cipro exposure under different growth temperatures. We focused in the integrative effects of temperature and Cipro on photosynthetic, respiratory and oxidative metabolism, as well as on the capacity of the plant to uptake the antibiotic from water. Moreover, since there is no consensus on how fluoroquinolones are absorbed into cells (Diver et al., 1990), we attempted to identify if Cipro uptake by this liverwort is a passive (by diffusion) or active process, and to relate the effects of the antibiotic and temperature on plant energetic metabolism and its ability to uptake Cipro.

## 2. Material and methods

### 2.1. Plant material and Cipro treatments

Plants of *Ricciocarpus natans* (L.) Corda. were acquired from Fundação Zoo-Botânica de Belo Horizonte (Belo Horizonte, Minas Gerais State, Brazil) and cultivated using Sterile CHU 10 medium (Chu, 1942). Prior to the initiation of the treatments (Cipro addition), the plants were washed three times in distilled water and transferred to 250 ml Erlenmeyer flasks containing CHU 10 medium, which were then stoppered with cotton (to minimize evaporation and avoid contamination). Flasks were kept for 15 days for acclimation in growth chambers where they were held at 25 °C, 30 °C or 35 °C (±2 °C) under a 12-h photoperiod (45 μmol photons

$m^{-2}$ $s^{-1}$, Philips T2 40 W/3 lamps). Experiments were performed using seven plants per acclimation flask, which were transferred to different Erlenmeyer flasks with CHU 10 medium (100 ml). Each group of plants acclimated to the above mentioned temperatures (25 °C, 30 °C or 35 °C) was treated with different concentrations of ciprofloxacin (0, 0.75, 1.05 and 2.25 mg $l^{-1}$) and arranged randomly in growth chambers at the same temperature as previous acclimation. Cipro concentrations were chosen based on the natural occurrence of the antibiotic in waters (Richardson et al., 2005; Lin and Tsai, 2009; Santos et al., 2010). The appropriated concentration of the antibiotic was applied directly to the CHU 10 medium priori the transference of plants to the Erlenmeyer flasks. All treatments were performed in triplicate. Analytical-grade ciprofloxacin (purity > 98%) was purchased from Sigma-Aldrich (Brazil) and was used in all the experiments.

### 2.2. Effects of Cipro on plant physiology

The effects of Cipro on plants were investigated after four days of exposure. To study the antibiotic's effects on photosynthesis, chlorophyll fluorescence in dark-adapted (15 min) fronds were evaluated using a Pulse-Amplitude Modulated (PAM) fluorometer (model PAM-2500, Walz, Effeltrich, Germany). The following parameters were evaluated: relative rate of electron transport through PSII (ETR) (Krall and Edwards, 1992), maximal photochemical efficiency of PSII ($F_v/F_m$) (Kitajima and Butler, 1975), non-photochemical quenching (NPQ) (Redondo-Gómez et al., 2008) and photochemical quenching (qP) (van Kooten and Snel, 1990). Fluorescence results of the 46 μmol photons $m^{-2}$ $s^{-1}$ light intensity (the light level most similar to growth conditions) were used to compare treatments.

Hydrogen peroxide ($H_2O_2$) content was determined according to Velikova et al. (2000) using ~0.01 g of plant tissue. To study antioxidant enzymes, ~0.1 g of plant tissue was ground in 1000 μl of extraction solution containing 100 mM potassium buffer (pH 7.8), 100 mM EDTA, 1 mM L-ascorbic acid, and 2% polyvinylpyrrolidone (PVP) (m/v). After determining total protein content (following the Bradford method), the activities of ascorbate peroxidase (APX; EC 1.11.1.11) (Nakano and Asada, 1981), catalase (CAT; EC1.11.1.6) (Aebi, 1984) and guaiacol peroxidase (GPX; EC 1.11.1.7) (Souza and MacAdam, 1998) were measured.

To study the effects of Cipro on mitochondrial electron transport activity, the enzymatic activities of Complexes I–IV were determined spectrophotometrically following the methods described in Huang et al. (2015). Intact mitochondria from plants were obtained following the method of Howell et al. (2006) with the modifications proposed by Murcha and Whelan (2015). Each assay was performed with 50 mg of protein. The intactness of isolated mitochondria (≥90% and ≥86% for all control and Cipro-treated samples, respectively) was measured by the organelle's latency, according to Burgess et al. (1985), using the activity of cytochrome *c* oxidase as marker enzyme in the presence (broken organelle) or absence (intact organelles) or Triton X-100. Membrane permeability was measured by the cell membrane stability (CMS) index in one frond per flask (corresponding to one replicate), using a conductivity meter (Gehaka CG1800) according to Sullivan and Ross (1979), and was calculated using the following formula:

$$\%CMS = \frac{1 - \frac{T1}{T2}}{1 - \frac{Ci}{C2}} * 100$$

where T and C are the conductivity of the Cipro treatment and control (without Cipro) samples, respectively. T1 and C1 represent electrolyte leakage (dS $m^{-1}$) after submerging the samples in deionized water at 25 °C for 4 h. T2 and C2 represent the total electrolyte concentrations as measured in samples heated in boiling water for 1 h and then cooled to room temperature. T and C are the conductivity of the treated and control samples, respectively. T1 and T2 correspond to the first and second solution conductivity determined for treated samples, and C1 and C2 are the respective values for the control. The results were expressed in % in relation to control plants (without Cipro treatment).

The quantity of ciprofloxacin in whole plants was determined in triplicate by high performance liquid chromatography coupled to a fluorescence detector. Cipro was extracted according to Palmada et al. (2000), with modifications by Migliore et al. (2003). After extraction, the samples were dried in a SpeedVac (RC1010, Thermo), and the residue suspended in a mobile phase (0.4% aqueous triethylamine pH 3.0, acetonitrile and methanol—75:10:15 v/v/v) of which 10 μl was injected into an HPLC (Shimadzu) equipped with a LC-10AD VP pump, a SIL-10AF auto injector, a SCL-10ASP system controller, and a Column Over CTO-10A VP. Evaluations were carried out following Zimmermann et al. (2016), using a fluorescence detector (RF-10A XL) and a C18 column (Discovery® HS C18 column 150 × 4.6 mm, particle size 5 μm), at a flow rate of 1.0 ml/min and with excitation/emission detection at 278/453 nm. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the method described by Mocak et al. (1997). The calculated LOD and LOQ were 5 ng $l^{-1}$ and 10 ng $l^{-1}$. Calibration curves of six points showed good linearity for the analyte ($r^2 = 0.99$; $P < 0.0001$) within the domain of expected sample concentration. Each batch of samples included three blanks, three standards, and three fortified samples. Recovery rates were higher than 85%.

### 2.3. Mechanism of Cipro uptake by plants

To investigate if Cipro uptake by plants is an active process, plants were cultivated in CHU 10 medium with the addition of 0 or 1.05 mg $l^{-1}$ of ciprofloxacin and 0 or 2 mM rotenone (an inhibitor of the mitochondrial electron transport chain) in a factorial scheme. Plants were kept under dark to minimize ATP production by metabolism other than from mitochondria (e. g. photosynthesis). After 30, 60, 120 and 240 min, plants were harvested. Cipro concentration in whole plants and mitochondria Complex I activity were evaluated by the methods previously described. Respiration rates were estimated using the closed system method. For each evaluation time, one plant was packed into a sealed glass tube, and the initial (ti) headspace $O_2$ level was measured using a portable gas analyzer (PBI Dansensor CheckPoint II), with 5 replicates. After the mentioned periods of incubation at the tested temperatures, final (tf) headspace $O_2$ levels were recorded. The respiration rate ($RO_2$) was calculated according to the following equation, and expressed as ml $O_2$ (consumed) $min^{-1}$

$$RO_2 = \frac{\Delta O_2}{\Delta t}$$

where $\Delta O_2$ is the $O_2$ difference (ml) measured in the system after a certain time ($\Delta t$), with $\Delta t$ = time (minutes) elapsed between measurements.

### 2.4. Statistical analyses

The results represent the averages of five replicates. Statistical analyses were performed using JMP software 10.0 (SAS Institute); the results were tested for normality (Shapiro-Wilk) and homoscedasticity (Brown-Forsythe) prior to statistical evaluation. Results from the "Effects of Cipro on plant physiology" section were

evaluated using two-way analysis of variance (full factorial for temperature x Cipro concentrations) and means were compared using the Tukey's test at a 5% level of probability (Table 1S). Results from the "Mechanism of Cipro uptake by plants" were submitted to multivariate repeated measures ANOVA. Time was used as the within-subject factor and temperature, Cipro concentration and rotenone as the main effects to analyze differences in the variable during the times of exposure to the treatments. Cipro, Temperature, Rotenone and the interactions between Cipro concentrations, Temperature and Rotenone were included within the model. The sphericity of the data was tested using Mauchly's criteria to determine whether the univariate F tests for the within-subject effects were valid. In cases of an invalid F, the Greenhouse-Geisser test was used to estimate epsilon (e). Contrast analyses were used when there were significant differences in the studied variables between treatments (Table 2S).

## 3. Results

### 3.1. Chlorophyll fluorescence

Maximal photochemical efficiency of PSII ($F_v/F_m$) and the electron transport through PSII (ETR) were higher in plants kept under 25 °C ($P < 0.001$, Fig. 1). ETR decreased in plants treated with Cipro, regardless of the concentration ($P < 0.001$). Meanwhile, $F_v/F_m$ decreased only in plants exposed to Cipro concentrations $\geq$1.05 mg $l^{-1}$ ($P < 0.001$, Fig. 1). Non-photochemical quenching (NPQ) and photochemical quenching (qP) were not significantly affected by treatments (Fig. 1; $P > 0.05$). Moreover, interactions between temperature and Cipro were not significant ($P > 0.05$) for all chlorophyll fluorescence parameters.

### 3.2. Oxidative stress markers

Significant interactions between temperature and Cipro concentration were observed for $H_2O_2$ concentration and antioxidant enzyme activity (APX, CAT and GPX) in the plants ($P < 0.05$; Fig. 2).

Regardless of the Cipro concentration, $H_2O_2$ concentrations were highest in plants kept under 20 °C (Fig. 2). With the exception for plants treated with 0.75 mg Cipro $l^{-1}$, greater $H_2O_2$ concentrations were observed in plants kept under 30 °C than under 25 °C. Moreover, regardless of the temperature, plants treated with concentrations $\geq$1.05 mg Cipro $l^{-1}$ exhibited greater $H_2O_2$ concentrations than their respective controls (0 mg Cipro $l^{-1}$).

APX and CAT activity was lowest in plants kept under 20 °C (Fig. 2). When treated with 0.75 and 2.25 mg Cipro $l^{-1}$, plants kept under 25 °C showed greater APX activity in relation to those grown under 30 °C. The treatment with 2.25 mg Cipro $l^{-1}$ resulted in decreased APX activity in plants kept under 25 °C and 30 °C, compared to plants treated with 0 and 0.75 mg Cipro $l^{-1}$. In contrast to APX, when treated with 2.25 mg Cipro $l^{-1}$, CAT activity was greater in plants kept under 30 °C compared to those under 25 °C. Moreover, under both 25 °C and 30 °C, Cipro treatment resulted in increased CAT activity relative to the control.

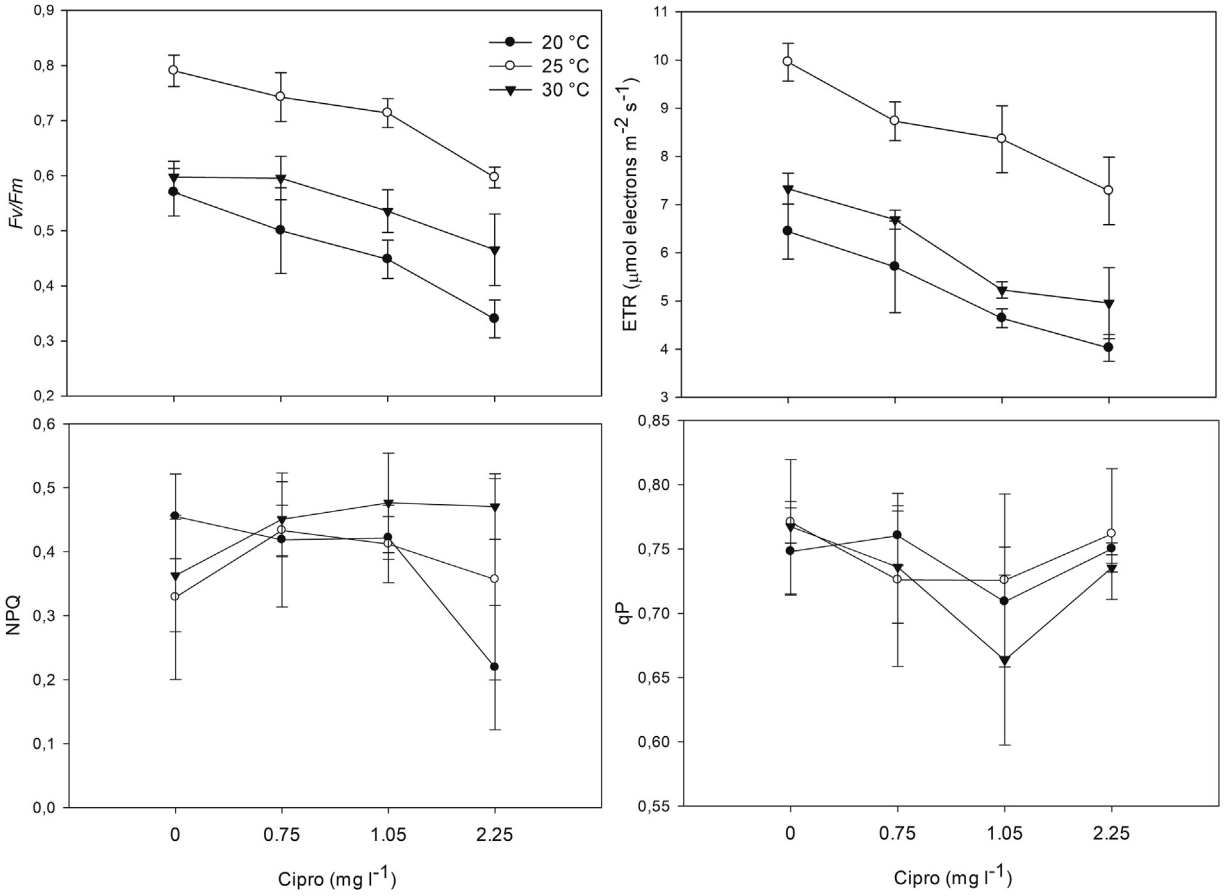



**Fig. 1.** Photosynthesis-related measurements [maximal photochemical efficiency of PSII ($F_v/F_m$), electron transport rate (ETR), non-photochemical quenching (NPQ) and photochemical quenching (qP)] in individual plants of *Ricciocarpus natans* exposed to increased ciprofloxacin concentrations (0, 0.75, 1.05 and 2.25 mg $l^{-1}$) and temperatures (20 °C, 25 °C and 30 °C) for 96 h. Bars represent means ± SD of five replicates.

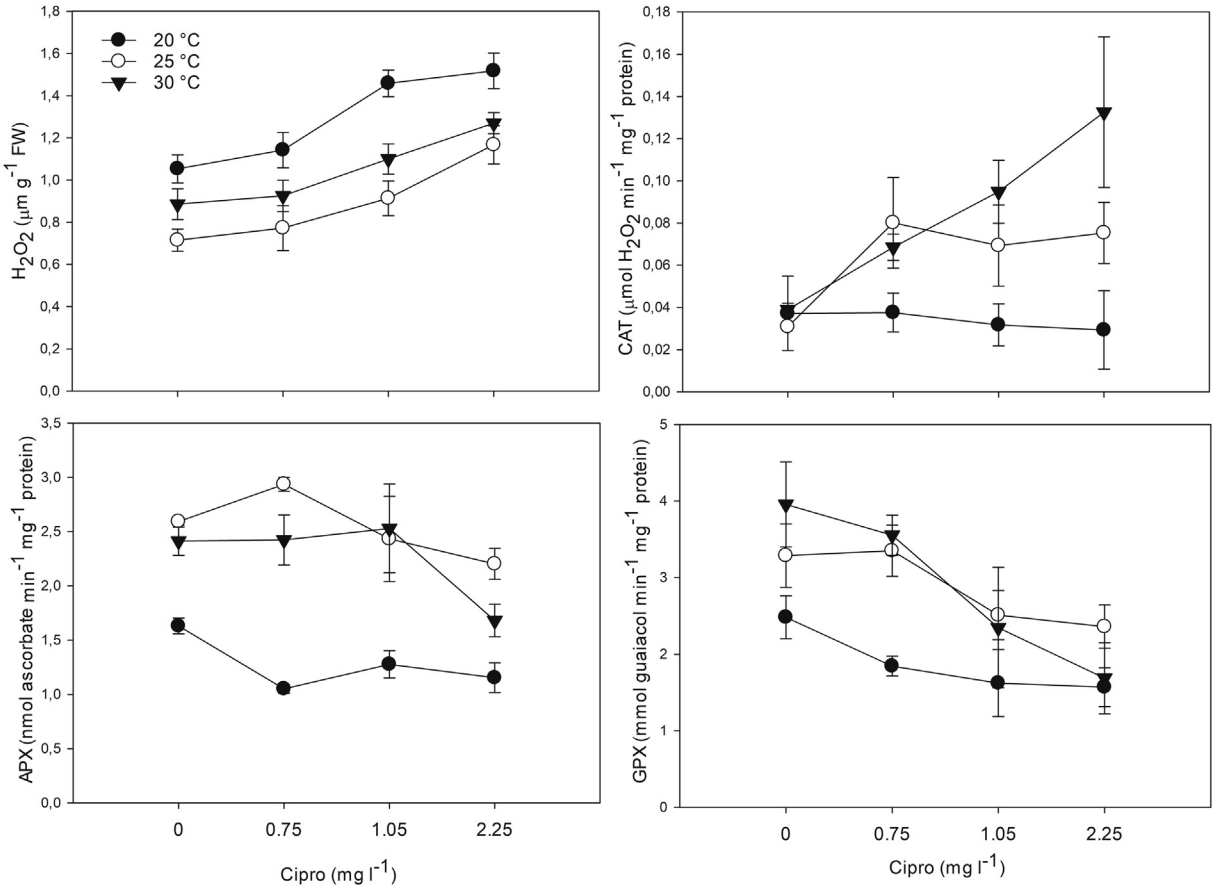



**Fig. 2.** Hydrogen peroxide ($H_2O_2$) and the activities of catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in *Ricciocarpus natans* plants exposed to increased ciprofloxacin concentrations (0, 0.75, 1.05 and 2.25 mg $l^{-1}$) and temperatures (20 °C, 25 °C and 30 °C) for 96 h. Bars represent means ± SD of five replicates.

GPX activity was greater in plants kept under 25 °C and 30 °C treated with 0 and 0.75 mg Cipro $l^{-1}$ than in those under 20 °C (Fig. 2). GPX activity decreased in plants kept under 30 °C treated with 1.05 and 2.25 mg Cipro $l^{-1}$.

### 3.3. Mitochondrial electron transport activity

A significant interaction ($P < 0.001$) between temperature and Cipro concentration was observed for all the mitochondria electron transport chain enzymes evaluated (Complexes I, II, III and IV; Fig. 3). In the absence of Cipro treatment (0 mg $l^{-1}$), enzyme activities increased as temperature increased. However, the activities of Complexes I, II and III were greatly decreased by Cipro in plants kept under 30 °C, mainly when compared to those kept under 25 °C. Within the same temperature, the negative effects of Cipro (0.75–2.25 mg $l^{-1}$) on the activities of Complexes I, III and IV did not differ significantly between the tested concentrations of the antibiotic. For Complex II, however, when kept under 20 °C and 30 °C, decreased activity was observed in plants treated with Cipro concentrations ≥1.05 mg $l^{-1}$, while enzyme activity decreased as the Cipro concentration increased in plants kept under 25 °C.

### 3.4. Membrane permeability and Cipro concentrations in plants

A significant interaction between temperature and Cipro concentration was observed for membrane permeability and Cipro concentration in plants ($P < 0.01$). Membrane permeability and Cipro concentration were highest in plants kept under 30 °C, particularly in those submitted to Cipro concentrations ≥1.05 mg $l^{-1}$ (Fig. 4). Moreover, membrane permeability and Cipro concentration were greater in plants kept under 25 °C than under 20 °C, when exposed to Cipro concentrations ≤1.05 mg $l^{-1}$ (Fig. 4). In plants treated with 2.25 mg Cipro $l^{-1}$, membrane permeability did not differ, but Cipro concentrations were higher in plants kept under 25 °C in relation to those under 20 °C (Fig. 4).

### 3.5. Mechanism of Cipro uptake by plants

Rotenone decreased $RO_2$ in plants regardless of Cipro concentration and exposure duration (Fig. 5). A significant interaction among temperature Cipro concentration and Rotenone was observed ($P < 0.01$). The $RO_2$ reduction by the inhibitor over time was greater as temperature increased and under Cipro presence.

A significant interaction among time of exposure, temperature, Cipro concentration and Rotenone addition was observed for Cipro concentration in plants ($P < 0.0001$). Regardless of the temperature, treatment with Rotenone resulted in decreased Cipro concentration in plants (Fig. 5). Moreover, as temperature increased, the Rotenone effect on Cipro concentration in plants increased over time ($P < 0.05$).

## 4. Discussion

Based on ETR, 25 °C appears to be optimal for growth of the liverwort. This temperature is also optimal for several species of tropical liverworts (Wagner et al., 2013), and many species show

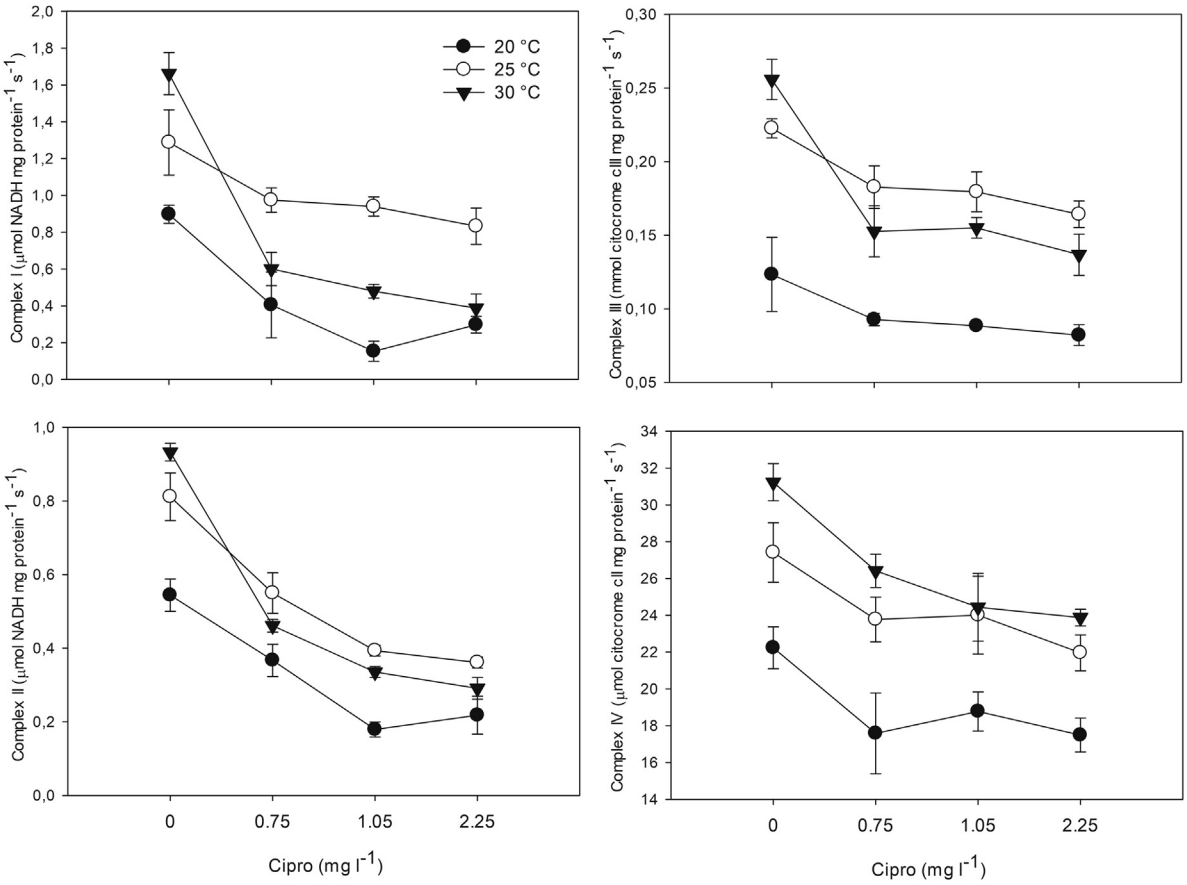



**Fig. 3.** Complex I (NADH:ubiquinone oxidoreductase), Complex II (succinate dehydrogenase), Complex III (Ubiquinol-cytochrome c reductase), and Complex IV (cytochrome c oxidase) activities in *Ricciocarpus natans* plants exposed to increased ciprofloxacin concentrations (0, 0.75, 1.05 and 2.25 mg $l^{-1}$) and temperatures (20 °C, 25 °C and 30 °C) for 96 h. Bars represent means ± SD of five replicates.

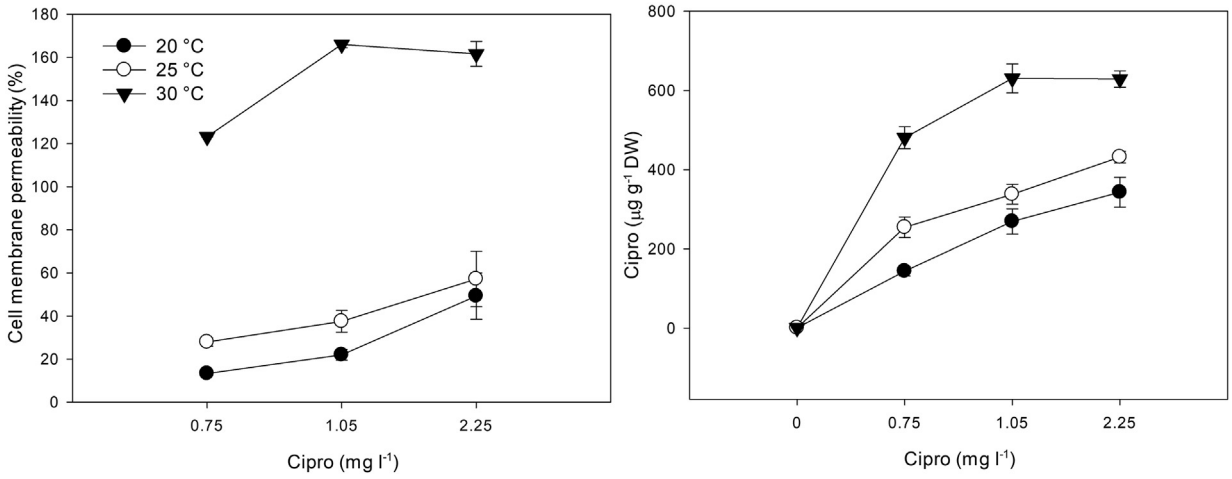



**Fig. 4.** Cell membrane permeability and ciprofloxaxin concentrations in *Ricciocarpus natans* plants exposed to increased ciprofloxacin concentrations (0, 0.75, 1.05 and 2.25 mg $l^{-1}$) and temperatures (20 °C, 25 °C and 30 °C) for 96 h. Bars represent means ± SD of five replicates.

decreases in photosynthesis and productivity under temperatures higher than 27 °C (Wagner et al., 2013). Photosynthesis rapidly rose in *R. natans* from 20 °C to 25 °C, but then declined at the highest temperature (30 °C). At low temperatures, the transport of associated components into cell membranes is prevented by their increased viscosity, which results in decreased rates of photosynthesis (Barber et al., 1984). Meanwhile, high temperatures largely limit photosynthesis in many bryophyte species, but the causes are not fully understood (He et al., 2016). It has been suggested that it is related to the activation state of RuBisCO, since RuBisCO activase is heat labile (He et al., 2016).

Temperatures required for optimal rates of photosynthesis

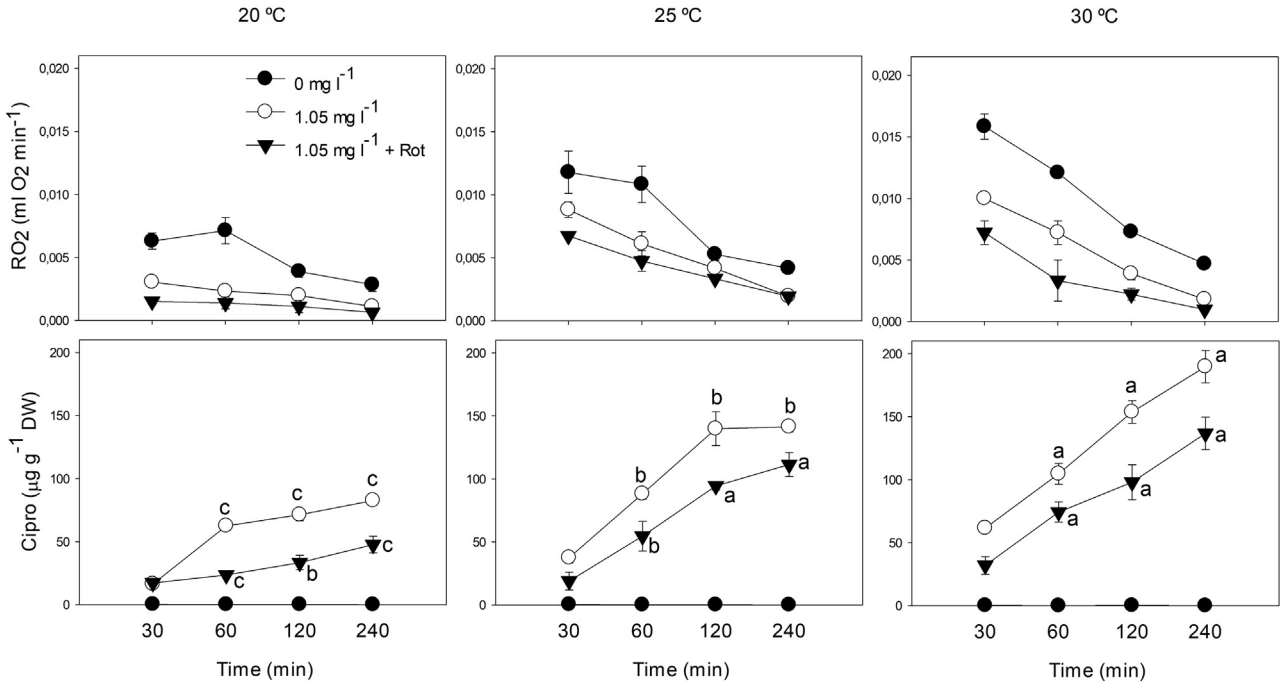



**Fig. 5.** Time-courses of respiration rate ($RO_2$) and ciprofloxacin concentrations in *Ricciocarpus natans* plants exposed to 0 or 1.05 mg ciprofloxacin $l^{-1}$, and treated with Rotenone (ROT) under increased temperatures (20 °C, 25 °C and 30 °C). Bars represent means ± SD of five replicates. Values followed by different letters, within the same ciprofloxacin concentration and time of evaluation, are significantly different ($P > 0.05$) by the contrast test.

(when carbon assimilation is maximal) are often lower than those of respiration (Raven and Geider, 1988). This means that moderate to high temperatures will constrain photosynthesis more than respiration. As a result, there is a proportionally higher rates of ATP and carbohydrate consumption than production (Raven and Geider, 1988) and respiration becomes the major load of photosynthesis (He et al., 2016). Indeed, the highest respiration rates (as evaluated by mitochondrial enzymes and $RO_2$ – Figs. 3 and 5) in control plants (0 mg $l^{-1}$) were observed in plants kept under 30 °C. This will invariably play a role in both the productivity and tolerance of plants exposed to Cipro.

Regardless of the temperature, exposure to Cipro concentrations ≥1.05 mg $l^{-1}$ resulted in decreased photosynthesis (ETR) in plants (Fig. 1), which could be related to the effects of the antibiotic on PSII structure. Maximal photochemical efficiency of PSII ($F_v/F_m$), which decreased in plants exposed to Cipro concentrations ≥ 1.05 mg $l^{-1}$ (Fig. 1), is a proxy of PSII integrity (Walter et al., 2003). Therefore, the decrease in photosynthesis upon exposure to the antibiotic in concentrations ≥1.05 mg $l^{-1}$ must be a result of damage to the PSII structure of plants. However, how does Cipro derange thylakoid structure in *R. natans*?

Although reactive oxygen species (ROS) are normally produced in chloroplasts, once accumulated, they can promote lipid peroxidation, affecting the integrity of thylakoid membranes and mainly PSII functioning (Gomes et al., 2017b). Moreover, ROS can disrupt the Calvin cycle by inhibiting the activity of related enzymes as well as the assembly and repair of PSII by suppressing the *de novo* synthesis of PSII-associated proteins (such as D1) (Foyer et al., 1994; Takahashi and Murata, 2008). Regardless of the temperature, $H_2O_2$ concentrations and $F_v/F_m$ were inversely related with decreased $F_v/F_m$ being observed in plants showing greater $H_2O_2$ concentrations (Figs. 1 and 2). Therefore, ROS may be responsible for damage to PSII and, thus, for decreasing photosynthesis in those plants treated with high concentrations of Cipro. Increased ROS concentrations followed by damage to PSII (decreased $F_v/F_m$) have been previously reported for plants under abiotic stress (Pandey et al., 2009; Gomes et al., 2017b).

To cope with ROS production, plants synthesize some important enzymes, such as ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (GPX), that are the components of the antioxidant system responsible for $H_2O_2$-scavenging. They are quite responsive to contaminants (Gomes et al., 2017a; Noctor et al., 2017) but, being enzymes, they are also under the control of temperature (Bicalho et al., 2017). Since CAT has lower affinity for $H_2O_2$ than peroxidases such as APX (Mittler, 2002), the maintenance of its activity under lowest temperature in plants without Cipro treatment was not sufficient to compensate for the decrease in APX and GPX activity in these plants, which resulted in $H_2O_2$ accumulation and related oxidative damage (to PSII, for instance). The enzymes APX and GPX have optimal activity under 28 °C (Nakano and Asada, 1981; Souza and MacAdam, 1998), but decreased activity of APX has been reported for temperatures ≤ 25 °C (Hull et al., 1997; Bicalho et al., 2017). Therefore, temperature limitations on the activity of $H_2O_2$-scavenging enzymes will consequently interfere with the performances of plants under Cipro stress. In plants treated with Cipro, in turn, CAT was observed to have an inverse pattern, with higher activities under increasing temperatures and Cipro concentrations. Once more, due to its lowest affinity for $H_2O_2$, increased CAT activity did not offset the lost peroxidase activities, leading to $H_2O_2$ accumulation. The mechanism by which Cipro can induce decreases in enzyme activities has yet to be elucidated. However, we propose that the increased oxidative status ($H_2O_2$ concentrations) upon decreased activity of APX and GPX can also result in disruption of these enzyme activities (Romero-Puertas et al., 2002; Gomes et al., 2014). ROS can react with proteins promoting protein carbonylation, an irreversible oxidative process in which the Lys, Arg, Pro and Thr side-chains are converted to aldehyde or keto groups (Romero-Puertas et al., 2002). While APX has affinity for $H_2O_2$ in µM range, CAT affinity is in the mM range (Mittler, 2002), which may be related to the lower sensitivity of CAT

to oxidative damage induced by $H_2O_2$ in relation to APX and GPX.

The enzymes APX and GPX were central to the control of ROS production under all temperatures, since the inhibition of these enzymes was followed by increased $H_2O_2$ concentrations in plants (Fig. 2). In contrast, CAT must be of great importance at temperatures of 25 °C and 30 °C [the closest temperature to that of optimal activity of CAT (Aebi, 1984)], mainly because its activity is not decreased by Cipro exposure. Moreover, the sharp increase in CAT activity in plants kept under 30 °C and exposed to 2.25 mg Cipro $l^{-1}$ assured $H_2O_2$ concentrations similar to those found in plants kept under 25 °C – the temperature at which the lowest $H_2O_2$ concentrations were reported for all other Cipro concentrations (Fig. 2). However, despite the increased activity of CAT in Cipro-exposed plants kept under 25 °C and 30 °C, it was not sufficient to prevent oxidative damage to PSII since $F_v/F_m$ decreased. We were, therefore, interested in understanding how ROS are produced in plants upon Cipro exposure.

Chloroplasts are great cellular sources of ROS. Electrons passing through the photosystems can be captured by the oxygen generated during photosynthesis, resulting in $O_2^-$ formation, which is then dismuted to $H_2O_2$ (Gill and Tuteja, 2010). Moreover, chloroplast possess ROS production centers, such as triplet chlorophyll and the ETC (Gill and Tuteja, 2010). In cases of decreased ETR, electrons harvested by chlorophylls drive ROS generation through triplet chlorophyll formation (Gill and Tuteja, 2010). In order to minimize the generation of the highly reactive $^1O_2$, plants dissipate excess light energy absorbed by PSII light-harvesting complexes (Demming-Adams and Adams, 2000), which can be measured during chlorophyll fluorescence kinetics as non-photochemical (NPQ) and photochemical energy dissipation (qP). Both parameters were not affected by temperature or Cipro exposure of plants, indicating that functional PSII is not responsible for ROS generation and that ROS might be formed outside of chloroplasts.

In addition to chloroplasts, the activity of the mitochondria of Complexes I, II and III is the major source of ROS in plants (Noctor et al., 2007; Quinlan et al., 2012; Moreno-Sánchez et al., 2013). Therefore, we were interested in investigating the activity of mitochondrial electron transport chain (ETC)-related enzymes. As expected, increasing temperatures favored the activity of these enzymes in plants without Cipro treatment (Fig. 3). Temperature modulates membrane fluidity and diffusion of electron carriers (Los and Murata, 2004). Rising temperatures decrease membrane viscosity, favoring the transport of associated components into the membranes (Barber et al., 1984) and increasing the intermolecular collision rates of electron carriers, decreasing their oxidation-reduction turnover times (Falkowski and Raven, 2013) – the reverse is observed at lower temperatures.

Although the activity of all the mitochondria ETC enzymes evaluated (Complexes I-IV) decreased upon Cipro exposure, Complexes I and II seemed to be the most affected. In Cipro-exposed plants, average reductions of 44%–68% and 50%–51% were observed in the activity of Complexes I and II, respectively, while average reductions of 24%–38% and 17%–22% were observed for Complexes III and IV, again respectively. Therefore, Cipro must act as a mitochondrial ETC-inhibitor (Gomes et al., 2017a).

During mitochondrial electron transport, Complexes I and II transfer electrons to the pool of oxidized ubiquinone (UQ), forming reduced ubiquinol ($UQH_2$), which is further oxidized in Complex III. $UQH_2$ is oxidated at the quinoloxidation (Qo) site of Complex III (Center P), thereby restoring UQ, which can also be reduced in the quinol-reducing (Qi) site (Center N) of Complex III – the Q-cycle, responsible for the proton pumping at Complex III (Crofts et al., 1999). To result in the concomitant reduction of the activity of Complexes I and II, Cipro may act as an inhibitor of Complex III activity, thus blocking the oxidation of quinol (at Center P) or the reduction of ubiquinol (at Center N). Complex II is relatively independent of Complex I, and therefore, decreases in Complex II activity is more likely to be related to the lack of succinate or free ubiquinone than to decreases in Complex I (Gomes et al., 2016). Since Complex I also used UQ as a substrate and less inhibition of Complex III activity was observed, we suggest that the activity of Complexes I and II must be more limited by substrate availability (UQ) than by Q-cycle activity in Complex III. Cipro, therefore, must act like myxothiazol, impairing the UQ-reaction site (Qo-site at Center P) of Complex III. This is also supported by the fact that when Rotenone (an inhibitor of Complex I) was used concomitantly with Cipro, decreased respiration rates ($RO_2$) were observed relative to plants only treated with Cipro (Fig. 4). Rotenone treatment will impair respiration upstream of the point of Cipro inhibition in the mitochondrial ETC, resulting in lesser mitochondrial activity (Gomes and Juneau, 2016).

Increases in ROS formation are common when mitochondrial ETC-inhibitors are used (Starkov and Fiskum, 2001; Li et al., 2003), and increased $H_2O_2$ production was observed in plants treated with Myxothiazol, which leads to the accumulation of unstable semiquinone at Qo-site and increases its side reaction with oxygen (Starkov and Fiskum, 2001). Similarly, by inhibiting Qo-site of Complex III, Cipro must lead to $H_2O_2$ production in exposed plants. Once produced, ROS can leave mitochondria and affect other cellular compartments. For example Gomes and Juneau (2016), showed by confocal microscopy for ROS location, that when *Lemna minor* was exposed to glyphosate, ROS were initially produced outside the chloroplast and then brought into by these organelles, causing oxidative damage to PSII.

Interestingly, under higher temperatures (30 °C), the deleterious effects of Cipro on the activity of mitochondrial ETC enzymes were accentuated. For instance, average reduction of 62% and 61% was observed for Complexes I and II, respectively, in Cipro-treated plants kept under 30 °C, while it was of 44% and 50% in plants kept under 25 °C. There are two hypotheses that explain these results: 1) decreased activity of mitochondrial enzymes under rising temperatures; or 2) increased concentrations of Cipro (which lead to greater inhibition of Complex III activity) in plants kept under 30 °C. Since higher temperatures favored enzyme activity (as seen in the control plants), we were interested in investigating the effects of temperature on Cipro-uptake by plants. Indeed, increased Cipro-uptake was observed in plants kept under 30 °C (Fig. 5), which explains the greater deleterious effects of Cipro on the activity of mitochondrial enzymes of those plants (Fig. 3). However, why were there increased concentrations of Cipro in plants under higher temperatures?

Membrane composition, fluidity and permeability are modulated by temperature, and increased fluidity and permeability of membranes under rising temperatures have been reported (Daniel et al., 1996; Los and Murata, 2004). Increased membrane fluidity and permeability may result in increased cell permeability to water pollutants (Gomes and Juneau, 2017), which may explain the greater concentration of Cipro in plants kept under 30 °C. One can argue that greater Cipro uptake must result in greater interference in mitochondrial ETC and increased oxidative status (ROS concentrations) in plants. However, it is important to note that those plants also had responses with regard to their antioxidant enzymes, reinforcing the role of these enzymes in controlling the deleterious effects of Cipro on plants.

The positive relationship between cell membrane permeability and Cipro concentration in plants attests to the possibility of a passive mechanism of uptake of this antibiotic. However, since Cipro acts on respiratory metabolism, we were interested in investigating whether part of Cipro uptake by plants must be an energy-dependent process. Therefore, plants were treated with

Cipro and an inhibitor of mitochondrial ETC (Rotenone) for 4 h, and respiration and Cipro uptake rates recorded (Fig. 5). Interestingly, when Complex I was inhibited by Rotenone, decreased Cipro concentrations were observed in plants in relative to those treated only with Cipro. As discussed earlier, Cipro may result in decreased ATP production by mitochondria by inhibiting the Qo-site of Complex III; however, Rotenone must have greater deleterious effects on respiration due to its upstream point of inhibition (Complex I). Since the effects of Rotenone treatment on $RO_2$ and Cipro concentration in plants were independent of temperature, we suggest that Cipro uptake by plants may be, at least in part, an active process dependent on ATP, as reported by Diver et al. (1990). However, the passive mechanism of Cipro-uptake must be predominant, since the greatest effects of Cipro on respiration (and therefore greater disruption in ATP production), and also increased permeability, was seen in plants kept under 30 °C.

## 5. Conclusion

Although the deleterious effects of Cipro on photosynthetic organisms has already been related to ROS formation, the present study proposes, for the first time to our knowledge, the specific site of Cipro-induced ROS formation in plants. Cipro appears to act as an inhibitor of the UQ-reaction site (Qo-site at Center P) of Complex III, blocking the oxidation of quinol (at Center P). This may lead to the accumulation of unstable semiquinone at the Qo-site and increase its side reaction with oxygen, resulting in the formation of ROS. The oxidative damage driven by ROS accumulation upon Cipro exposure is, in turn, responsible for decreased photosynthesis in plants. Increased temperature favored Cipro uptake, as well as its deleterious effects on mitochondrial activity, however, it also favors the activity of antioxidant enzymes, preventing exacerbated deleterious effects of Cipro on plants. Finally, the uptake of Cipro by plants appears to be mainly a passive process, although some uptake must be driven by an energy-consuming process. In this context, *R. natans* must be considered for Cipro reclamation programs, particularly when considering global warming, as this liverwort exhibited high Cipro-tolerance, the capacity for accumulation and increased uptake rates of the antibiotic with rising temperatures (from 20 to 30 °C).

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgements

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the study grant awarded to the first and second authors, and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity scholarship awarded to Q.S. Garcia. We also thank Dr. Marien Rodrigues Ribeiro da Cunha from Fundação Ezequiel Dias for technical support on Cipro evaluations; and Prof Alessandra Giani from UFMG for the help and support in growing the plants.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.03.048.

## References

Aebi, H., 1984. Catalase in vitro. Meth. Enzymol. 105, 121–126.
Aristilde, L., Melis, A., Sposito, G., 2010. Inhibition of photosynthesis by a fluoroquinolone antibiotic. Environ. Sci. Technol. 44, 1444–1450.
Barber, J., Ford, R.C., Mitchell, R.A.C., Millner, P.A., 1984. Chloroplast thylakoid membrane fluidity and its sensitivity to temperature. Planta 161, 375–380.
Bedard, J., Wong, S., Byan, L.E., 1987. Accumulation of enoxacin by *Escherichia coli* and *Bacillus subtilis*. Antimicrob. Agents Chemother. 31, 1348–1354.
Bicalho, E.M., Gomes, M.P., Rodrigues-Junior, A.G., Oliveira, T.G.S., de Almeida Gonçalves, C., Fonseca, M.B., Garcia, Q.S., 2017. Integrative effects of zinc and temperature on germination in *Dimorphandra wilsonii* rizz.: implications of climate changes. Environ. Toxicol. Chem. 9999, 1–7.
Bradley, P.M., Journey, C.A., Romanok, K.M., et al., 2017. Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in U.S. Streams. Environ. Sci. Technol. 7b00012 acs.est.
Brighty, K.E., Gootz, T.D., 2000. In: Andriole, V.T. (Ed.), The Quinolones. Academic Press, San Diego, pp. 34–98.
Burgess, N., Beakes, G., Thomas, D., 1985. Separation of mitochondria from microbodies of *Pisum sativum* L. cv. Alaska cotyledons. Planta 166, 151–155.
Chalifour, A., Juneau, P., 2011. Temperature-dependent sensitivity of growth and photosynthesis of *Scenedesmus obliquus*, *Navicula pelliculosa* and two strains of *Microcystis aeruginosa* to the herbicide atrazine. Aquat. Toxicol. (Amst.) 103, 9–17.
Chapman, J.S., Georgopapadakou, N.H., 1988. Routes of quinolone permeation in *Escherichia coli*. Antimicrob. Agents Chemother. 32, 438–442.
Chiodi Boudet, L., Escalante, A., Von Haeften, G., Moreno, V., Gerpe, M., 2011. Assessment of heavy metal accumulation in two aquatic macrophytes: a field study. J. Braz. Soc. Ecotoxicol. 6, 57–64.
Chu, S.P., 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. J. Ecol. 30, 284–325.
Cohen, S.P., Hooper, D.C., Wolfson, J.S., Souza, K.S., McMurry, L.M., Levy, S.B., 1988. Endogenous active efflux of norfloxacin in susceptible *Escherichia coli*. Antimicrob. Agents Chemother. 32, 1187–1191.
Crofts, A.R., Barquera, B., Gennis, R.B., Kuras, R., Guergova-Kuras, M., Berry, E.A., 1999. Mechanism of ubiquinol oxidation by the bc(1) complex: different domains of the quinol binding pocket and their role in the mechanism and binding of inhibitors. Biochemistry 38, 15807–15826.
Daniel, R.M., Dines, M., Peatach, H.H., 1996. The denaturation and degradation of stable enzymes at high temperatures. Biochem. J. 317, 1–11.
Demming-Adams, B., Adams, W., 2000. Photosynthesis: harvesting sunlight safety. Nature 403, 371–374.
Diver, J.M., Piddock, L.J.V., Wise, R., 1990. The accumulation of five quinolone antibacterial agents by *Escherichia coli*. J. Antimicrob. Chemother. 25, 319–333.
Falkowski, P.G., Raven, J.A., 2013. In: Falkowski, P.G., Raven, J.A. (Eds.), Aquatic Photosynthesis. Princeton University Press.
Folt, C.L., Chen, C.Y., Moore, M., Burnaford, J., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. 44, 854–877.
Foyer, C.H., Leiandais, M., Kunert, K.J., 1994. Photooxidative stress in plants. Physiol. Plantarum 92, 696–717.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Girardi, C., Greve, J., Lamshöft, M., Fetzer, I., Miltner, A., Schäffer, A., Kästner, M., 2011. Biodegradation of ciprofloxacin in water and soil and its effects on the microbial communities. J. Hazard Mater. 198, 22–30.
Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ. Sci. Technol. 37, 3243–3249.
Gomes, M.P., Cruz, F.V.S., Borges, F.V., Fonseca, M.B., Garcia, Q.S., 2016. Modulation of *Dimorphandra wilsonii* Rizz. seed germination through H2O2 production in response to Zn interference of the mitochondrial electron transport chain. Environ. Exp. Bot. 128, 51–58.
Gomes, M.P., Gonçalves, C.A., de Brito, J.C.M., Souza, A.M., da Silva Cruz, F.V., Bicalho, E.M., Figueredo, C.C., Garcia, Q.S., 2017a. Ciprofloxacin induces oxidative stress in duckweed (*Lemna minor* L.): implications for energy metabolism and antibiotic-uptake ability. J. Hazard Mater. 328, 140–149.
Gomes, M.P., Juneau, P., 2016. Oxidative stress in duckweed (*Lemna minor* L.) induced by glyphosate: is the mitochondrial electron transport chain a target of this herbicide? Environ. Pollut. 218, 402–409.
Gomes, M.P., Juneau, P., 2017. Temperature and light modulation of herbicide toxicity on algal and cyanobacterial physiology. Front. Environ. Sci. 5, 50. https://doi.org/10.3389/fenvs.2017.00050.
Gomes, M.P., Le Manac'h, S.G., Hénault-Éthier, L., Labrecque, M., Lucotte, M., Juneau, P., 2017b. Glyphosate-dependent inhibition of photosynthesis in willow. Front. Plant Sci. 8, 207.
Gomes, M.P., Soares, A.M., Garcia, Q.S., 2014. Phosphorous and sulfur nutrition modulate antioxidant defenses in *Myracrodruom urundeuva* plants exposed to arsenic. J. Hazard Mater. 276, 97–104.
He, X., He, K.S., Hyvönen, J., 2016. Will bryophytes survive in a warming world? Perspectives in Plant Ecology. Evol. Syst.s 19, 49–60.
Howell, K.A., Millar, A.H., Whelan, J., 2006. Ordered assembly of mitochondria during rice germination begins with pro-mitochondrial structures rich in components of the protein import apparatus. Plant Mol. Biol. 60, 201–223.
Huang, S., Lee, C.P., Millar, A.H., 2015. Activity assay for plant mitochondrial enzymes. In: Whelan, J., Murcha, M.W. (Eds.), Plant Mitochondria: Methods and Protocols. Springer Protocols, pp. 139–149.
Hull, M.R., Long, S.P., Jahnke, L.S., 1997. Instantaneous and developmental effects of low temperature on the catalytic properties of antioxidant enzymes in two *Zea* species. Funct. Plant Biol. 24, 337–343.

Kitajima, M., Butler, W.L.W., 1975. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochem. Biophys. Acta 376, 105–115.
Knutti, R., Rogelj, J., Sedlacek, J., Fischer, E.M., 2016. A scientific critique of the two-degree climate change target. Nat. Geosci. 9, 13–18.
van Kooten, O., Snel, J., 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25, 147–150.
Kozieł, R., Zabłocki, K., Duszyn, J., 2006. Calcium signals are affected by ciprofloxacin as a consequence of reduction of mitochondrial DNA content in Jurkat cells. Antimicrob. Agents Chemother. 50, 1664–1671.
Krall, J.P., Edwards, G.E., 1992. Relationship between photosystem II activity and $CO_2$ fixation in leaves. Physiol. Plant. 86, 180–187.
Lawrence, J.W., Claire, D.C., Weissig, V., Rowe, T.C., 1996. Delayed cytotoxicity and cleavage of mitochondrial DNA in ciprofloxacin-treated mammalian cells. Mol. Pharmacol. 50, 1178–1188.
Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J.A., Robinson, J.P., 2003. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. J. Biol. Chem. 278, 8516–8525.
Lin, A.Y.C., Tsai, Y.T., 2009. Occurrence of pharmaceuticals in Taiwan's surface waters, impact of waste streams from hospitals and pharmaceutical production facilities. Sci. Total Environ. 407, 3793–3802.
Los, D.A., Murata, N., 2004. Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta Biomembr. 1666, 142–157.
Lowes, D.A., Wallace, C., Murphy, M.P., Webster, N.R., Galley, H.F., 2009. The mitochondria targeted antioxidant MitoQ protects against fluoroquinolone-induced oxidative stress and mitochondrial membrane damage in human Achilles tendon cells. Free Radic. Res. 43, 323–328.
Martínez-Carballo, E., González-Barreiro, C., Scharf, S., Gans, O., 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ. Pollut. 148, 570–579.
McClellan, K., Halden, R.U., 2010. Pharmaceuticals and personal care products in archived U.S. biosolids from the 2001 EPA national sewage sludge survey. Water Res. 44, 658–668.
Migliore, L., Cozzolino, S., Fiori, M., 2003. Phytotoxicity to and uptake of enrofloxacin in crop plants. Chemosphere 52, 1233–1244.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.
Mocak, J., Bonde, A.M., Mitchell, S., Scollary, G., 1997. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: application to voltammetric and stripping techniques. Pure Appl. Chem. 69, 297–328.
Moreno-Sánchez, R., Hernández-Esquivel, L., Rivero-Segura, N.A., Marín-Hernández, A., Neuzil, J., Ralph, S.J., Rodríguez-Enríquez, S., 2013. Reactive oxygen species are generated by the respiratory complex II – evidence for lack of contribution of the reverse electron flow in complex I. FEBS J. 280, 927–938.
Murcha, M.W., Whelan, J., 2015. In: Whelan, J., Murcha, M.W. (Eds.), Isolation of Intact Mitochondria from the Model Plant Species Arabidopsis thaliana and Oryza Sativa.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Noctor, G., De Paepe, R., Foyer, C.H., 2007. Mitochondrial redox biology and homeostasis in plants. Trends Plant Sci. 12, 125–134.
Noctor, G., Reichheld, J.-P., Foyer, C.H., 2017. ROS-related redox regulation and signaling in plants. Semin. Cell Dev. Biol. https://doi.org/10.1016/j.semcdb.2017.07.013 pii: S1084-9521(17)30246-X.
Palmada, J., March, R., Torroella, E., Espigol, C., Baleri, T., 2000. Determination of enrofloxacin and its active metabolite (ciprofloxacin) at the residue level in broiler muscle using HPLC with fluorescence detector. In: Van Ginkel, L.A., Ruiter, A. (Eds.), Proceedings of Euroresidue IV. Residues of Veterinary Drugs in Food. Wijk Bij Duurstede: ADDIX, pp. 822–826.
Pandey, V., Dixit, V., Shyam, R., 2009. Chromium effect on ROS generation and detoxification in pea (*Pisum sativum*) leaf chloroplasts. Protoplasma 236, 85–95.
Quinlan, C.L., Orr, A.L., Perevoshchikova, I.V., Treberg, J.R., Ackrell, B.A., Brand, M.D., 2012. Mitochondrial Complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions. J. Biol. Chem. 287, 27255–27264.
Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. New Phytol. 110, 441–461.
Redondo-Gómez, S., Mateos-Naranjo, E., Cambrollé, J., Luque, T., Figueroa, M.E., Davy, A.J., 2008. Carry-over of differential salt tolerance in plants grown from dimorphic seeds of *Suaeda splendens*. Ann. Bot. 102, 103–112.
Richardson, B.J., Lam, P.K.S., Martin, M., 2005. Emerging chemicals of concern, pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. Mar. Pollut. Bull. 50, 913–920.
Romero-Puertas, M.C., Palma, J.M., Gómez, M., Del Río, L.A., Sandalio, L.M., 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant. Cell Environ. 25, 677–686.
Santos, L.H.M.L.M., Araujo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard Mater. 175, 45–95.
SFT, 2007. Occurencee of selected pharmaceuticals in wastewater effluents from hospitals (Ullevål and Rikshospitalet) and VEAS wastewater treatment works. Norwegian Pollutant Control Authority SFT Report, TA-2246/2007.
Sharma, A., Sachdeva, S., 2015. Cadmium toxicity and its phytoremediation a review. Int. J. Sci. Eng. Res. 6, 395–405.
Sobrino, C., Neale, P.J., 2007. Short-term and long-term effects of temperature on photosynthesis in the diatom *Thalassiosira pseudonana* under uvr exposures. J. Phycol. 43, 426–436.
Souza, I.R.P., MacAdam, J.W., 1998. A transient increase in apoplastic peroxidase activity preceds decrease in elongation rate of B73 maize (*Zea mays* L.) leaf blades. Physiol. Plantarum 104, 556–562.
Starkov, A.A., Fiskum, G., 2001. Myxothiazol induces $H_2O_2$ production from mitochondrial respiratory chain. Biochem. Biophys. Res. Commun. 281, 645–650.
Sullivan, C., Ross, W., 1979. Selecting for drought and heat resistance sorghum. In: Mussell, H., Taples, T. (Eds.), Stress Physiology in Crops Plants. John Willey and Sons, USA, pp. 264–281.
Takahashi, S., Murata, N., 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13, 178–182.
Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, 59–66.
Wagner, S., Zotz, G., Allen, N.S., Bader, M.Y., 2013. Altitudinal changes in temperature responses of net photosynthesis and dark respiration in tropical bryophytes. Ann. Bot. 111, 455–465.
Walter, A., Rascher, U., Osmond, B., 2003. Transitions in photosynthetic parameters of midvein and interveinal regions of leaves and their importance during leaf growth and development. Plant Biol. 6, 184–191.
Zimmermann, E.S., Torres, B.G.S., Dalla Costa, T., 2016. Validation of a sensitive HPLC/fluorescence method for assessment of ciprofloxacin levels in plasma and prostate microdialysate samples from rats. Biomed. Chromatogr. 30, 330–336.